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(54) Title: NOVEL MUSCARINIC ACETYLCHOLINE RECEPTOR ANTAGONISTS

(57) Abstract: Muscarinic Acetylcholine receptor antagonists and methods of using them are provided.



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Novel Muscarinic Acetylcholine Receptor Antagonists

FIELD OF THE INVENTION

This invention relates to novel derivatives of cyclic amines, pharmaceutical
5 compositions, processes for their preparation, and use thereof in treating muscarinic
acetylcholine receptor mediated diseases.

BACKGROUND OF THE INVENTION

Acetylcholine released from cholinergic neurons in the peripheral and central
10 nervous systems affects many different biological processes through interaction with two
major classes of acetylcholine receptors – the nicotinic and the muscarinic acetylcholine
receptors. Muscarinic acetylcholine receptors (mAChRs) belong to the superfamily of G-
protein coupled receptors that have seven transmembrane domains. There are five
subtypes of mAChRs, termed M₁-M₅, and each is the product of a distinct gene. Each of
15 these five subtypes displays unique pharmacological properties. Muscarinic acetylcholine
receptors are widely distributed in vertebrate organs, and these receptors can mediate
both inhibitory and excitatory actions. For example, in smooth muscle found in the
airways, bladder and gastrointestinal tract, M₃ mAChRs mediate contractile responses.
For review, please see {Brown 1989 247 /id}.

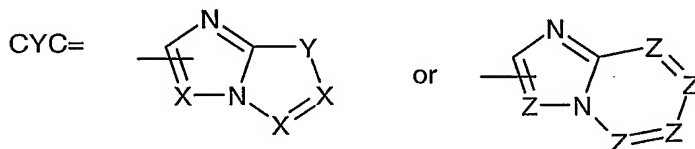
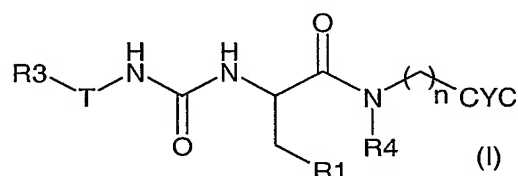
20 Muscarinic acetylcholine receptor dysfunction has been noted in a variety of
different pathophysiological states. For instance, in asthma and chronic obstructive
pulmonary disease (COPD), inflammatory conditions lead to loss of inhibitory M₂
muscarinic acetylcholine autoreceptor function on parasympathetic nerves supplying the
pulmonary smooth muscle, causing increased acetylcholine release following vagal nerve
25 stimulation. This mAChR dysfunction results in airway hyperreactivity mediated by
increased stimulation of M₃ mAChRs{Costello, Evans, et al. 1999 72 /id}{Minette,
Lammers, et al. 1989 248 /id}. Similarly, inflammation of the gastrointestinal tract in
inflammatory bowel disease (IBD) results in M₃ mAChR-mediated hypermotility {Oprins,
Meijer, et al. 2000 245 /id}. Incontinence due to bladder hypercontractility has also been
30 demonstrated to be mediated through increased stimulation of M₃ mAChRs {Hegde &
Eglen 1999 251 /id}. Thus the identification of subtype-selective mAChR antagonists
may be useful as therapeutics in these mAChR-mediated diseases.

Despite the large body of evidence supporting the use of anti-muscarinic receptor therapy for treatment of a variety of disease states, relatively few anti-muscarinic compounds are in use in the clinic. Thus, there remains a need for novel compounds that are capable of causing blockade at M₃ mAChRs. Conditions associated with an increase
 5 in stimulation of M₃ mAChRs, such as asthma, COPD, IBD and urinary incontinence would benefit by compounds that are inhibitors of mAChR binding.

SUMMARY OF THE INVENTION

This invention relates to compounds of Formula I

10



wherein

Y is S, O; or NR₄

X is N, or CR₅, provided that the number of N at the X value cannot exceed 2;

15 Z is N, or CR₅, provided that the number N at the Z value cannot exceed 3;

N is an integer from 0 to 3;

R₁ is selected from the group consisting of C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, unsubstituted or substituted phenyl, or unsubstituted or substituted phenyl C₁-C₃ lower alkyl; wherein, when

20 substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₈ alkoxy, halo, hydroxy, amino, cyano, trifluoromethyl, C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, phenyl and phenyl C₁-C₃ lower alkyl;

T is selected from the group consisting of thiophene, furan, thiazole, isothiazole, pyrrole,
 25 imidazole, pyrazole and para-substituted phenyl which may be substituted by radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, trifluoromethyl,

C₁-C₄ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl and phenyl;

R₃ is selected from the group consisting of COR₆, COOR₆, OSO₂R₆, N(R₇)SO₂R₆, CONR₆R₇, NR₆R₇, OCOR₆, OCONR₆R₇, NHCOR₆, N(R₇)COR₆, NHCOOR₆ and NHCONR₆R₇;

R₄ is selected from the group consisting of hydrogen, C₁-C₃ alkyl and allyl;

R₅ is selected from the group consisting of hydrogen, C₁-C₃ alkyl, C₁-C₃ alkenyl, , halo, NR₄, OR₄, CN, NO₂, and trifluoromethyl;

R₆ is selected from the group consisting of substituted or unsubstituted C₁-C₈ branched or unbranched alkyl, C₃-C₁₂ cycloalkyl, C₃-C₁₂ cycloalkenyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, phenyl, and phenyl C₁-C₃ lower alkyl wherein, when substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, cyano, nitro, trifluoromethyl, and C₁-C₃ branched or unbranched alkyl;

R₇ is selected from the group consisting of hydrogen, C₁-C₄ alkyl and allyl.

The present invention includes all hydrates, solvates, complexes and prodrugs of the compounds of this invention. Prodrugs are any covalently bonded compounds that release the active parent drug according to Formula I **in vivo**. If a chiral center or another form of an isomeric center is present in a compound of the present invention, all forms of such isomer or isomers, including enantiomers and diastereomers, are intended to be covered herein. Inventive compounds containing a chiral center may be used as a racemic mixture, an enantiomerically enriched mixture, or the racemic mixture may be separated using well-known techniques and an individual enantiomer may be used alone. In cases in which compounds have unsaturated carbon-carbon double bonds, both the cis (Z) and trans (E) isomers are within the scope of this invention. In cases wherein compounds may exist in tautomeric forms, such as keto-enol tautomers, each tautomeric form is contemplated as being included within this invention whether existing in equilibrium or predominantly in one form.

The meaning of any substituent at any one occurrence in Formula I or any subformula thereof is independent of its meaning, or any other substituent's meaning, at any other occurrence, unless specified otherwise.

Abbreviations and symbols commonly used in the peptide and chemical arts are used herein to describe the compounds of the present invention. In general, the amino

acid abbreviations follow the IUPAC-IUB Joint Commission on Biochemical Nomenclature as described in **Eur. J. Biochem.**, 158, 9 (1984).

The term "C₁-C₈ alkyl" and "C₁-C₆ alkyl" is used herein includes both straight or branched chain radicals of 1 to 6 or 8 carbon atoms. By example this term includes, but is not limited to methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, *tert*-butyl, pentyl, hexyl, heptyl, octyl and the like. "Lower alkyl" has the same meaning as C₁-C₈ alkyl.

Herein "C₁-C₈ alkoxy" includes straight and branched chain radicals of the likes of -O-CH₃, -O-CH₂CH₃, and the n-propoxy, isopropoxy, n-butoxy, sec-butoxy, isobutoxy, *tert*-butoxy, pentoxy, and hexoxy, and the like.

"C₃-C₈-cycloalkyl" as applied herein is meant to include substituted and unsubstituted cyclopropane, cyclobutane, cyclopentane and cyclohexane, and the like.

"Halogen" or "halo" means F, Cl, Br, and I.

The preferred compounds of Formula I include those compounds wherein, Y is S, O; or NR₄

X is N, or CR₅, provided that the number of N at the X value cannot exceed 2;

Z is N, or CR₅, provided that the number of N at the Z position cannot exceed 3;

N is an integer from 0-3;

R₁ is selected from the group consisting of C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, unsubstituted or substituted phenyl, or unsubstituted or substituted phenyl C₁-C₃ lower alkyl; wherein, when substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₈ alkoxy, halo, hydroxy, amino, cyano, trifluoromethyl, C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, phenyl and phenyl C₁-C₃ lower alkyl;

T is selected from the group consisting of thiophene, furan, thiazole, isothiazole, pyrrole, imidazole, pyrazole or para-substituted phenyl which may be substituted by radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, trifluoromethyl,

C₁-C₄ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl and phenyl;

R₃ is selected from the group consisting of COR₆, COOR₆, OSO₂R₆, N(R₇)SO₂R₆, CONR₆R₇, NR₆R₇, OCOR₆, OCONR₆R₇, NHCOR₆, N(R₇)COR₆, NHCOOR₆ and NHCONR₆R₇;

R4 is selected from the group consisting of hydrogen, C1-C3 alkyl and allyl;

R5 is selected from the group consisting of hydrogen, C1-C3 alkyl, C1-C3 alkenyl, , halo, NR4, OR4, CN, NO₂, and trifluoromethyl;

5 R6 is selected from the group consisting of substituted or unsubstituted C₁-C₈ branched or unbranched alkyl, C₃-C₁₂ cycloalkyl, C₃-C₁₂ cycloalkenyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, phenyl, and phenyl C1-C3 lower alkyl wherein, when substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, cyano, nitro, trifluoromethyl, and C₁-C₃ branched or unbranched alkyl;

10 R7 is selected from the group consisting of: hydrogen, C1-C4 alkyl or allyl.

More preferred are those compounds where:

Y is S, or O;

X is CR5;

15 Z is CR5;

n is 1 or 2;

R1 is selected from the group consisting of unsubstituted or substituted phenyl wherein, when substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₈ alkoxy, halo, hydroxy, amino, cyano, trifluoromethyl, C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, phenyl and phenyl C1-C3 lower alkyl;

20

T is selected from the group consisting of thiophene, furan, or para-substituted phenyl which may be substituted by radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, trifluoromethyl, C₁-C₄ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl and phenyl.

25

R3 is selected from the group consisting of COOR6, OSO₂R6, N(R7)SO₂R6, and CONR6R7;

R4 is selected from the group consisting of hydrogen, C1-C3 alkyl and allyl;

30 R5 is selected from the group consisting of hydrogen, C1-C3 alkyl, C1-C3 alkenyl, , halo, NR4, OR4, CN, NO₂, and trifluoromethyl;

R6 is selected from the group consisting of substituted or unsubstituted C₁-C₈ branched or unbranched alkyl, C₃-C₁₂ cycloalkyl, C₃-C₁₂ cycloalkenyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, phenyl, or phenyl C1-C3 lower alkyl wherein, when substituted, a

group is substituted by one or more radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, cyano, nitro, trifluoromethyl, and C₁-C₃ branched or unbranched alkyl;

R₇ is selected from the group consisting of hydrogen, C₁-C₃ alkyl and allyl.

5 Even more preferred are those compounds where:

Y is S;

X is CR₅;

Z is CR₅;

N is 1;

10 R₁ is selected from the group consisting of unsubstituted or substituted phenyl wherein, when substituted, a group is substituted at the meta or para position by one radical selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, cyano, nitro, trifluoromethyl, and C₁-C₃ branched or unbranched alkyl;

T is of thiophene or para-substituted phenyl;

15 R₃ is selected from the group consisting of COOR₆, OSO₂R₆, and N(R₇)SO₂R₆;

R₄ is hydrogen;

R₅ is hydrogen;

R₆ is selected from the group consisting of substituted or unsubstituted C₁-C₈ branched or unbranched alkyl, C₃-C₉ cycloalkyl, C₃-C₉ cycloalkenyl, C₃-C₈ cycloalkylmethyl, and

20 C₃-C₈ alkenyl;

The preferred compounds are selected from the group consisting of:

N-[[(4-[(1-methylethyl)amino]sulfonyl)phenyl)amino]carbonyl]-*N*-[(2-methylimidazo[2,1-*b*][1,3]thiazol-6-yl)methyl]-L-tyrosinamide;

25 Methyl 5-([[(1*S*)-1-[(4-hydroxyphenyl)methyl]-2-[(2-methylimidazo[2,1-*b*][1,3]thiazol-6-yl)methyl]amino]-2-oxoethyl)amino]carbonyl)amino)-2-thiophenecarboxylate;

Cyclohexyl 5-([[(1*S*)-1-[(4-[(1,1-dimethylethyl)oxy]phenyl)methyl]-2-[(2-methylimidazo[2,1-*b*][1,3]thiazol-6-yl)methyl]amino]-2-oxoethyl) amino]carbonyl)amino)-2-thiophenecarboxylate; or a pharmaceutically acceptable salts thereof.

30

Methods of Preparation

Preparation

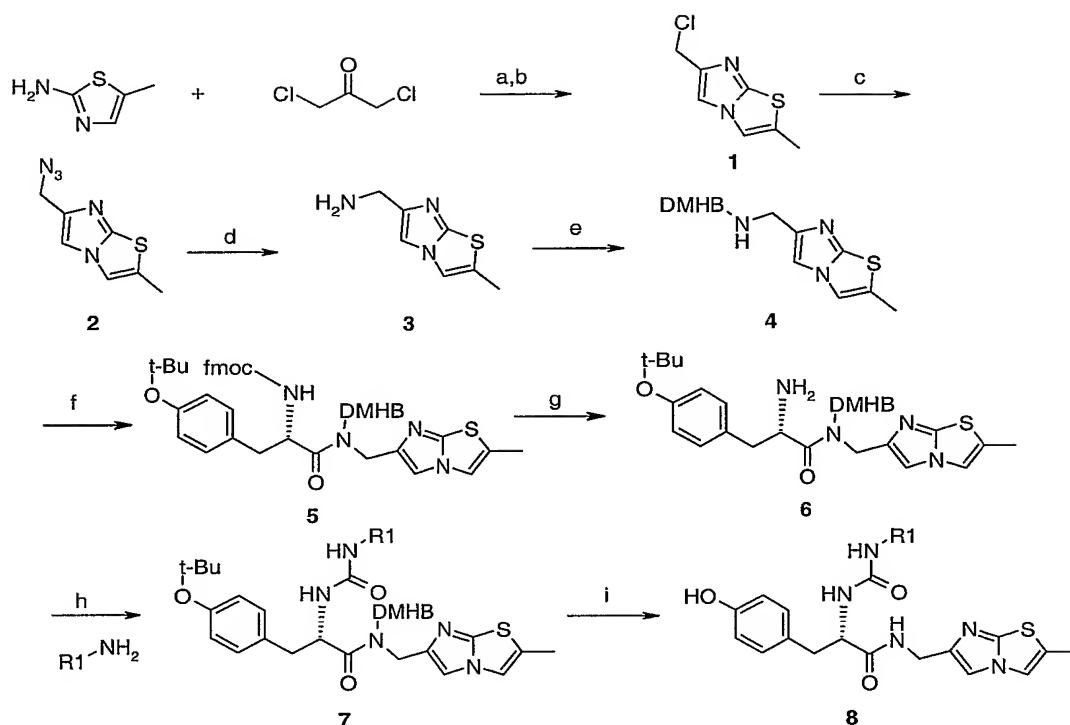
The compounds of Formula (I) may be obtained by applying synthetic procedures, some of which are illustrated in the Schemes below. The synthesis provided for these Schemes

is applicable for producing compounds of Formula (I) having a variety of different R1, R3, R4, R5 and R6, which are reacted, employing substituents which are suitable protected, to achieve compatibility with the reactions outlined herein. Subsequent deprotection, in those cases, then affords compounds of the nature generally disclosed. While some Schemes are shown with specific compounds, this is merely for illustration purpose only.

Preparation 1

Compounds of formula **8** can be prepared according to the general schemes (1, 2 and 3) depicted below. Scheme 1 showed the solid phase synthesis. 2-Amino-5-methylthiazole was treated with sodium bromide and dichloroacetone to provide 6-(chloromethyl)-2-methylimidazo[2,1-*b*][1,3]thiazole **1**, which was reacted with sodium azide to form 6-(azidomethyl)-2-methylimidazo[2,1-*b*][1,3]thiazole **2**. The azide **2** was converted to amine **3** by hydrogenation via 10% palladium on carbon. Resin-bound amine **4** was prepared by reductive amination of 2,6-dimethoxy-4-polystyrenebenzyloxy-benzaldehyde (DMHB resin) with amine **3**. Reaction of **4** with Fmoc-protected amino acid, followed by removal of the protecting group, provided resin-bound intermediate **6**. The amines were coupled with resin-bound intermediate **6** to afford the corresponding resin-bound ureas **7**. The resin was then cleaved by 50% trifluoroacetic acid in dichloromethane to afford targeted compounds **8** (Scheme 1).

Scheme 1



Conditions: a) NaBr, rt, 14 hrs. b) 110 °C for 1 hrs. c) NaN₃, DMSO, 65 °C d) 10% palladium on carbon, H₂, rt; e) 2,6-dimethoxy-4-polystyrenebenzyloxy-benzaldehyde (DMHB resin),

- 5 Na(OAc)₃BH, diisopropylethylamine, 10% acetic acid in 1-methyl-2-pyrrolidinone, rt; f) Fmoc-protected amino acids, 1,3-diisopropylcarbodiimide, 1-hydroxy-7-azabenzotriazole, 1-methyl-2-pyrrolidinone, rt; g) 20% piperidine in 1-methyl-2-pyrrolidinone, rt; h) 4-nitrobenzene chloroformate, R₁NH₂, diisopropylethylamine, N,N-dimethyl formamide, dichloromethane, rt; i) 50% trifluoroacetic acid in dichloromethane, rt.

10

SYNTHETIC EXAMPLES

The following examples are provided as illustrative of the present invention but not limiting in any way:

15

Example 1

Preparation of methyl 5-({[(((1S)-1-[(4-hydroxyphenyl)methyl]-2-[(2-methylimidazo[2,1-b][1,3]thiazol-5-yl)methyl]amino)-2-oxoethyl)amino]carbonyl}amino)-2-thiophenecarboxylate

20

Sodium bromide (7.2 g, 69.98 mmol) and dichloroacetone (4.88 g, 38.43 mmol) were added to 2-Amino-5-methylthiazole (4.0 g, 35.04 mmol) in ethyl acetate (140 mL).

The reaction mixture was stirred at room temperature for 14 hours. Significant amount of solid precipitated out and filtered off. The solid was dissolved in acetic acid (250 mL) and heated to 110 °C for 1 hour. The reaction mixture was cooled to room temperature overnight. Significant amount of solid precipitated out again. The solid was filtered off,
5 washed with acetic acid (75 mL), acetone (200 mL), diethyl ether (200 mL) and air dried overnight to afford 6-(chloromethyl)-2-methylimidazo[2,1-*b*][1,3]thiazole **1** (4.86 g, 74.2%).
LC/MS 151.2 [M+H-Cl]⁺ Rt, 0.72min.

6-(Chloromethyl)-2-methylimidazo[2,1-*b*][1,3]thiazole **1** (4.47 g, 23.82 mmol) was dissolved in DMSO (50 mL). Sodium azide (1.55 g, 23.82 mmol) was added. The reaction
10 mixture was heated to 65 °C overnight. Water (200 mL) was added after the reaction mixture was cooled to room temperature. The mixture was extracted with ethyl acetate (3x 150 mL). The combined organic phase was washed with brine(150 mL), dried over MgSO₄, concentrated and purified with combiflash eluting with (100% methylene chloride to 5:95% methylene chloride verse ethyl acetate) to afford 6-(azidomethyl)-2-
15 methylimidazo[2,1-*b*][1,3]thiazole **2** (1.64 g, 35%).
LCMS (ESI) 194.0 [M+H]⁺ Rt, 1.24 min.

6-(Azidomethyl)-2-methylimidazo[2,1-*b*][1,3]thiazole **2** (1.64 g, 8.45 mmol) was dissolved in methanol (50 mL). 10% Palladium on carbon (0.42 g) was added. The reaction mixture was hydrogenated at 1 atmosphere overnight to afford 1-(2-
20 methylimidazo[2,1-*b*][1,3]thiazol-6-yl)methanamine **3** (1.30 g, 97%) after filtration and concentration.
LCMS (ESI) 335.2 [2M+H]⁺ Rt, 1.16 min.

To a mixture of 5.78 g (8.68 mmol, 1.50 mmol/g) of 2,6-dimethoxy-4-
25 polystyrenebenzyloxy-benzaldehyde (DMHB resin) in 100 mL of 10% acetic acid in anhydrous 1-methyl-2-pyrrolidinone was added of 1-(2-methylimidazo[2,1-*b*][1,3]thiazol-6-yl)methanamine **3** (2.9 g, 17.36 mmol) and 6.05 mL (34.72 mmol) of diisopropylethyl amine, followed by addition of sodium triacetoxymethylborohydride (7.38 g, 34.72 mmol). After the resulting mixture was shaken at rt for 72 h, the resin was washed with DMF (3 x 250
30 mL), CH₂Cl₂/MeOH (1:1, 3 x 250 mL) and MeOH (3 x 250 mL). The resulting resin was dried in vacuum oven at 35 °C for 24 h.

To a mixture of the above resin (8.68 mmol) in 100 mL of anhydrous 1-methyl-2-pyrrolidinone was added 11.6 g (25.14 mmol) of Fmoc-Try(tBu)-OH and 1.18 g (8.68 mmol) of 1-hydroxy-7-azabenzotriazole, followed by addition of 5.51 mL (34.72 mmol) of

1,3-diisopropylcarbodiimide. After the resulting mixture was shaken at rt for 24 h, the resin was washed with DMF (3 x 50 mL), CH₂Cl₂/MeOH (1:1, 3 x 50 mL) and MeOH (3 x 50 mL). The resulting resin **5** was dried in vacuum oven at 35 °C for 24 h. An analytical amount of resin was cleaved with 50% trifluoroacetic acid in dichloroethane for 2 h at rt.

5 The resulting solution was concentrated *in vacuo*: MS (ESI) 553.6 [M+H-tBu]⁺. Rt, 1.78 min.

The above resin **5** (8.68 mmol) was treated with 100 mL of 20% piperidine in anhydrous 1-methyl-2-pyrrolidinone solution. After the mixture was shaken at room
10 temperature for 15 min, the solution was drained and another 100 mL of 20% piperidine in anhydrous 1-methyl-2-pyrrolidinone solution was added. The mixture was shaken at rt for another 15 min. The solution was drained and the resin was washed with DMF (3 x 50 mL), CH₂Cl₂/MeOH (1:1, 3 x 50 mL) and MeOH (3 x 50 mL). The resulting resin **6** was dried in vacuum oven at 35 °C for 24 h. An analytical amount of resin was cleaved
15 with 50% trifluoroacetic acid in dichloroethane for 2 h at rt. The resulting solution was concentrated *in vacuo*: MS (ESI) 331 [M+H-tBu]⁺ Rt, 1.06 min.

5-Nitro-2-thiophenecarboxylic acid (2.0 g, 11.55 mmol), K₂CO₃ (4.78 g, 34.65 mmol) and methyl iodide (6.56 g, 46.22mmol) were suspended in DMF (20 mL). The reaction mixture was stirred at room temperature for 6 hours. The reaction mixture was
20 quenched with water (25 mL), extracted with ethyl acetate (100 mLx3) and dried over MgSO₄. The crude methyl 2-nitrothiophenecarboxylate was obtained after concentration, which was used in the next step without further purification.

To methyl 5-nitro-2-thiophenecarboxylate (11.55 mmol) in ethyl alcohol (20 mL) was added palladium on carbon (10%, 1 g). The reaction mixture was hydrogenated with an
25 atmosphere of H₂ overnight. Methyl 5-amino-2-thiophenecarboxylate (1.67 g, 92%) was obtained after filtration and concentration.

LCMS (ESI) 157 [M+H]⁺.

To a mixture of 350 mg (2.23 mmol) methyl 5-amino-2-thiophenecarboxylate in 10mL of anhydrous dichloromethane was added 448 mg (2.23 mmol) 4-
30 nitrobenzylchloroformate. The reaction mixture was stirred at room temperature for half an hour and concentrated. Diisopropylethylamine (1.09 mL, 6.22 mmol), DMHB resin bound *N*-[(2-methylimidazo[2,1-*b*][1,3]thiazol-6-yl)methyl]-L-tyrosinamide(300 mg, 0.24 mmol) **6** and dimethyl formamide (10 mL) were added to reaction mixture and shaken overnight. The resin was washed with CH₂Cl₂ (3 x 10 mL), CH₂Cl₂/MeOH (1:1, 3 x 10

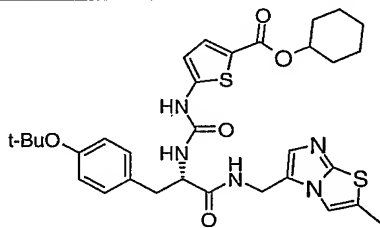
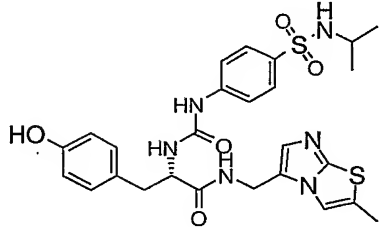
mL), MeOH (3 x 10 mL) and CH₂Cl₂ (3 x 10mL). The resulting resin was dried in vacuum oven at 35 °C for 24 h.

The dry resin was treated with 2 mL of 50% trifluoroacetic acid in dichloromethane at rt for 2h. After the cleavage solution was collected, the resin was treated with another 2 mL of 50% trifluoroacetic acid in dichloromethane at rt for 10min. The combined cleavage solutions were concentrated *in vacuo*. The residue was purified using a Gilson semi-preparative HPLC system with a YMC ODS-A (C-18) column 50 mm by 20 mm ID, eluting with 10% B to 90% B in 3.2 min, hold for 1 min where A = H₂O (0.1% trifluoroacetic acid) and B = CH₃CN (0.1% trifluoroacetic acid) pumped at 25 mL/min, to produce methyl 5-(((1*S*)-1-[(4-hydroxyphenyl)methyl]-2-[[[(2-methylimidazo[2,1-*b*][1,3]thiazol-5-yl)methyl]amino]-2-oxoethyl)amino]carbonyl]amino)-2-thiophene carboxylate (white powder, 7 mg, 5.7% over 5 steps).

MS (ESI) 514.4 [M+H]⁺ Rt 1.51 min

Proceeding in a similar manner as described in example 1, but replacing methyl 5-amino-2-thiophenecarboxylate with the appropriate amines, compounds listed in Tables 1 were prepared.

Table 1

Example	R1	MS [M] ⁺	Rt (min)
2		638.4	2.17
3		571.4	1.46

BIOLOGICAL EXAMPLES

The inhibitory effects of compounds at the M₃ mAChR of the present invention are determined by the following *in vitro* and *in vivo* assays:

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Analysis of Inhibition of Receptor Activation by Calcium Mobilization:

1) 384-well FLIPR assay

A CHO (chinese hamster ovary) cell line stably expressing the human M₃ muscarinic acetylcholine receptor is grown in DMEM plus 10% FBS, 2 mM Glutamine and 200 ug/ml G418. Cells are detached for maintenance and for plating in preparation for assays using either enzymatic or ion chelation methods. The day before the FLIPR (fluorometric imaging plate reader) assay, cells are detached, resuspended, counted, and plated to give 20,000 cells per 384 well in a 50 ul volume. The assay plates are black clear bottom plates, Becton Dickinson catalog number 35 3962. After overnight incubation of plated cells at 37 degrees C in a tissue culture incubator, the assay is run the next day. To run the assay, media are aspirated, and cells are washed with 1x assay buffer (145mM NaCl, 2.5mM KCl, 10mM glucose, 10mM HEPES, 1.2 mM MgCl₂, 2.5mM CaCl₂, 2.5mM probenecid (pH 7.4.) Cells are then incubated with 50ul of Fluo-3 dye (4uM in assay buffer) for 60 – 90 minutes at 37 degrees C. The calcium- sensitive dye allows cells to exhibit an increase in fluorescence upon response to ligand via release of calcium from intracellular calcium stores. Cells are washed with assay buffer, and then resuspended in 50ul assay buffer prior to use for experiments. Test compounds and antagonists are added in 25 ul volume, and plates are incubated at 37 degrees C for 5 -30 minutes. A second addition is then made to each well, this time with the agonist challenge, acetylcholine. It is added in 25 ul volume on the FLIPR instrument. Calcium responses are measured by changes in fluorescent units. To measure the activity of inhibitors / antagonists, acetylcholine ligand is added at an EC₈₀ concentration, and the antagonist IC₅₀ can then be determined using dose response dilution curves. The control antagonist used with M₃ is atropine.

30

2) 96-well FLIPR assay

Stimulation of mAChRs expressed on CHO cells were analyzed by monitoring receptor-activated calcium mobilization as previously described . CHO cells stably expressing M₃ mAChRs were plated in 96 well black wall/clear bottom plates. After 18 to 24 hours, media was aspirated and replaced with 100 µl of load media (EMEM with Earl's salts,

35

0.1% RIA-grade BSA (Sigma, St. Louis MO), and 4 μ M Fluo-3-acetoxymethyl ester fluorescent indicator dye (Fluo-3 AM, Molecular Probes, Eugene, OR) and incubated 1 hr at 37° C. The dye-containing media was then aspirated, replaced with fresh media (without Fluo-3 AM), and cells were incubated for 10 minutes at 37° C. Cells were then washed 3 times and incubated for 10 minutes at 37° C in 100 μ l of assay buffer (0.1% gelatin (Sigma), 120 mM NaCl, 4.6 mM KCl, 1 mM KH_2PO_4 , 25 mM NaHCO_3 , 1.0 mM CaCl_2 , 1.1 mM MgCl_2 , 11 mM glucose, 20mM HEPES (pH 7.4)). 50 μ l of compound (1×10^{-11} – 1×10^{-5} M final in the assay) was added and the plates were incubated for 10 min. at 37° C. Plates were then placed into a fluorescent light intensity plate reader (FLIPR, Molecular Probes) where the dye loaded cells were exposed to excitation light (488 nm) from a 6 watt argon laser. Cells were activated by adding 50 μ l of acetylcholine (0.1-10 nM final), prepared in buffer containing 0.1% BSA, at a rate of 50 μ l/sec. Calcium mobilization, monitored as change in cytosolic calcium concentration, was measured as change in 566 nm emission intensity. The change in emission intensity is directly related to cytosolic calcium levels. The emitted fluorescence from all 96 wells is measured simultaneously using a cooled CCD camera. Data points are collected every second. This data was then plotting and analyzed using GraphPad PRISM software.

Methacholine-induced bronchoconstriction

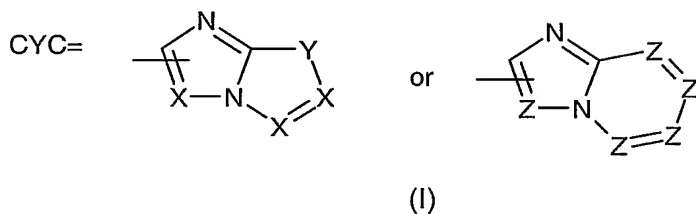
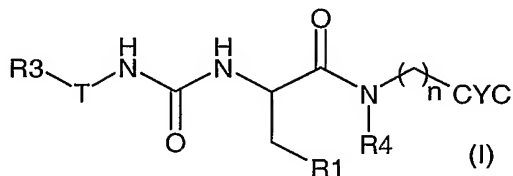
Airway responsiveness to methacholine was determined in awake, unrestrained BalbC mice ($n = 6$ each group). Barometric plethysmography was used to measure enhanced pause (Penh), a unitless measure that has been shown to correlate with the changes in airway resistance that occur during bronchial challenge with methacholine. Mice were pretreated with 50 μ l of compound (0.003-10 μ g/mouse) in 50 μ l of vehicle (10% DMSO) intranasally, and were then placed in the plethysmography chamber. Once in the chamber, the mice were allowed to equilibrate for 10 min before taking a baseline Penh measurement for 5 minutes. Mice were then challenged with an aerosol of methacholine (10 mg/ml) for 2 minutes. Penh was recorded continuously for 7 min starting at the inception of the methacholine aerosol, and continuing for 5 minutes afterward. Data for each mouse were analyzed and plotted by using GraphPad PRISM software.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A compound according to formula (I) below:



wherein

Y is S, O; or NR₄;

X is N, or CR₅, provided that the number of N at the X value cannot exceed 2;

10 Z is N, or CR₅, provided that the number N at the Z value cannot exceed 3;

N is an integer from 0 to 3;

R₁ is selected from the group consisting of C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, unsubstituted or substituted phenyl, or unsubstituted or substituted phenyl C₁-C₃ lower alkyl; wherein, when

15 substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₈ alkoxy, halo, hydroxy, amino, cyano, trifluoromethyl, C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, phenyl and phenyl C₁-C₃ lower alkyl;

20 T is selected from the group consisting of thiophene, furan, thiazole, isothiazole, pyrrole, imidazole, pyrazole and para-substituted phenyl which may be substituted by radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, trifluoromethyl, C₁-C₄ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl and phenyl;

25 R₃ is selected from the group consisting of COR₆, COOR₆, OSO₂R₆, N(R₇)SO₂R₆, CONR₆R₇, NR₆R₇, OCOR₆, OCONR₆R₇, NHCOR₆, N(R₇)COR₆, NHCOOR₆ and NHCONR₆R₇;

R₄ is selected from the group consisting of hydrogen, C₁-C₃ alkyl and allyl;

R5 is selected from the group consisting of hydrogen, C1-C3 alkyl, C1-C3 alkenyl, , halo, NR4, OR4, CN, NO₂, and trifluoromethyl;

R6 is selected from the group consisting of substituted or unsubstituted C₁-C₈ branched or unbranched alkyl, C₃-C₁₂ cycloalkyl, C₃-C₁₂ cycloalkenyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, phenyl, and phenyl C1-C3 lower alkyl wherein, when substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, cyano, nitro, trifluoromethyl, and C₁-C₃ branched or unbranched alkyl;

R7 is selected from the group consisting of hydrogen, C1-C4 alkyl and allyl;

10 or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 Y is S, O; or NR4

X is N, or CR5, provided that the number of N at the X value cannot exceed 2;

Z is N, or CR5, provided that the number of N at the Z position cannot exceed 3;

15 N is an integer from 0-3;

R1 is selected from the group consisting of C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, unsubstituted or substituted phenyl, or unsubstituted or substituted phenyl C1-C3 lower alkyl; wherein, when substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₈ alkoxy, halo, hydroxy, amino, cyano, trifluoromethyl, C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, phenyl and phenyl C1-C3 lower alkyl;

20

T is selected from the group consisting of thiophene, furan, thiazole, isothiazole, pyrrole, imidazole, pyrazole or para-substituted phenyl which may be substituted by radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, trifluoromethyl, C₁-C₄ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl and phenyl;

25

R3 is selected from the group consisting of COR6, COOR6, OSO₂R6, N(R7)SO₂R6, CONR6R7, NR6R7, OCOR6, OCONR6R7, NHCOR6, N(R7)COR6, NHCOOR6 and NHCONR6R7;

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R4 is selected from the group consisting of hydrogen, C1-C3 alkyl and allyl;

R5 is selected from the group consisting of hydrogen, C1-C3 alkyl, C1-C3 alkenyl, , halo, NR4, OR4, CN, NO₂, and trifluoromethyl;

R6 is selected from the group consisting of substituted or unsubstituted C₁-C₈ branched or unbranched alkyl, C₃-C₁₂ cycloalkyl, C₃-C₁₂ cycloalkenyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, phenyl, and phenyl C₁-C₃ lower alkyl wherein, when substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, cyano, nitro, trifluoromethyl, and C₁-C₃ branched or unbranched alkyl;

R7 is selected from the group consisting of: hydrogen, C₁-C₄ alkyl or allyl; or a pharmaceutically acceptable salt thereof.

10 3. A compound according to claim 1 wherein:

Y is S, or O;

X is CR₅;

Z is CR₅;

n is 1 or 2;

15 R1 is selected from the group consisting of unsubstituted or substituted phenyl wherein, when substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₈ alkoxy, halo, hydroxy, amino, cyano, trifluoromethyl, C₁-C₈ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl, phenyl and phenyl C₁-C₃ lower alkyl;

20 T is selected from the group consisting of thiophene, furan, or para-substituted phenyl which may be substituted by radicals selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, trifluoromethyl, C₁-C₄ branched or unbranched alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkyl lower alkyl and phenyl.

25 R3 is selected from the group consisting of COOR₆, OSO₂R₆, N(R₇)SO₂R₆, and CONR₆R₇;

R4 is selected from the group consisting of hydrogen, C₁-C₃ alkyl and allyl;

R5 is selected from the group consisting of hydrogen, C₁-C₃ alkyl, C₁-C₃ alkenyl, , halo, NR₄, OR₄, CN, NO₂, and trifluoromethyl;

30 R6 is selected from the group consisting of substituted or unsubstituted C₁-C₈ branched or unbranched alkyl, C₃-C₁₂ cycloalkyl, C₃-C₁₂ cycloalkenyl, C₃-C₈ cycloalkyl lower alkyl, C₃-C₈ alkenyl, phenyl, or phenyl C₁-C₃ lower alkyl wherein, when substituted, a group is substituted by one or more radicals selected from the group consisting of C₁-C₃

alkoxy, halo, hydroxy, amino, cyano, nitro, trifluoromethyl, and C₁-C₃ branched or unbranched alkyl;

R₇ is selected from the group consisting of hydrogen, C₁-C₃ alkyl and allyl; or a pharmaceutically acceptable salt thereof.

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4. A compound according to claim 1 Y is S;

X is CR₅;

Z is CR₅;

N is 1;

10 R₁ is selected from the group consisting of unsubstituted or substituted phenyl wherein, when substituted, a group is substituted at the meta or para position by one radical selected from the group consisting of C₁-C₃ alkoxy, halo, hydroxy, amino, cyano, nitro, trifluoromethyl, and C₁-C₃ branched or unbranched alkyl;

T is of thiophene or para-substituted phenyl;

15 R₃ is selected from the group consisting of COOR₆, OSO₂R₆, and N(R₇)SO₂R₆;

R₄ is hydrogen;

R₅ is hydrogen;

R₆ is selected from the group consisting of substituted or unsubstituted C₁-C₈ branched or unbranched alkyl, C₃-C₉ cycloalkyl, C₃-C₉ cycloalkenyl, C₃-C₈ cycloalkylmethyl, and

20 C₃-C₈ alkenyl;

or a pharmaceutically acceptable salt thereof.

5. A compound according to claim 1 selected from the group consisting of:

25 *N*-{[(4-[(1-methylethyl)amino]sulfonyl)phenyl]amino}carbonyl}-*N*-[(2-methylimidazo[2,1-*b*][1,3]thiazol-6-yl)methyl]-L-tyrosinamide;

Methyl 5-((((1*S*)-1-[(4-hydroxyphenyl)methyl]-2-[(2-methylimidazo[2,1-*b*][1,3]thiazol-6-yl)methyl]amino)-2-oxoethyl)amino)carbonyl)amino)-2-thiophenecarboxylate; and

30 Cyclohexyl 5-((((1*S*)-1-[(4-[(1,1-dimethylethyl)oxy]phenyl)methyl]-2-[(2-methylimidazo[2,1-*b*][1,3]thiazol-6-yl)methyl]amino)-2-oxoethyl) amino)carbonyl)amino)-2-thiophenecarboxylate;

or a pharmaceutically acceptable salt thereof.

6. A pharmaceutical composition for the treatment of muscarinic acetylcholine receptor mediated diseases comprising a compound according to claim 1 and a

35 pharmaceutically acceptable carrier thereof.

7. A method of inhibiting the binding of acetylcholine to its receptors in a mammal in need thereof comprising administering a safe and effective amount of a compound according to claim 1.

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8. A method of treating a muscarinic acetylcholine receptor mediated disease, wherein acetylcholine binds to said receptor, comprising administering a safe and effective amount of a compound according to claim 1.

10 9. A method according to claim 8 wherein the disease is selected from the group consisting of chronic obstructive lung disease, chronic bronchitis, asthma, chronic respiratory obstruction, pulmonary fibrosis, pulmonary emphysema and allergic rhinitis.

15 10. A method according to claim 9 wherein administration is via inhalation via the mouth or nose.

11. A method according to claim 10 wherein administration is via a medicament dispenser selected from a reservoir dry powder inhaler, a multi-dose dry powder inhaler or a metered dose inhaler.

20

12. A method according to claim 11 wherein the compound is administered to a human and has a duration of action of 12 hours or more for a 1 mg dose.

25 13. A method according to claim 12 wherein the compound has a duration of action of 24 hours or more.

14. A method according to claim 13 wherein the compound has a duration of action of 36 hours or more.